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O 40 PERFORMANCE OF ANTI-*SALMONELLA* LACTIC ACID BACTERIA IN THE PORCINE INTESTINE

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Summary: Of five anti-*Salmonella* porcine cultures administered to pigs at 10^{10} cfu/day, two *Lactobacillus murinus* strains demonstrated superior survival during gastrointestinal transit. Both were detected at 10^{7}-10^{8} cfu/g faeces which was higher (P<0.05) than *Pediococcus pentosaceus* DPC6006 (10^{5} cfu/g). One *Lb. murinus* strain was also excreted at higher numbers (P<0.05) than either *Lb. salivarius* DPC6005 or *Lb. pentosus* DPC6004 (both 10^{6} cfu/g). The *Lb. murinus* strains persisted in both the faeces and the caecum for at least 9 days post-administration. Animals fed a combination of all five strains at 10^{10} cfu/day excreted 10^{7} cfu/g of the administered strains, which was higher (P<0.05) than only *P. pentosaceus* DPC6006. Randomly amplified polymorphic DNA (RAPD) PCR analysis revealed that both *Lb. murinus* strains predominated in the faeces of these animals during administration, while post-administration, both *Lb. murinus* strains and *Lb. pentosus* DPC6004 were recovered from the faeces and the caecum while *P. pentosaceus* DPC6006 was only detected in the caecum. After 21 days of culture administration, faecal *Enterobacteriaceae* counts were reduced in pigs fed *Lb. salivarius* DPC6005, *P. pentosaceus* DPC6006, *Lb. pentosus* DPC6004 and the culture mix, though not significantly. Overall, the porcine intestinal isolates offer potential as probiotics for enteropathogen reduction in pigs; possibly as a combination due to strain variation.

Keywords: probiotic, pigs, gastrointestinal tract, *Lactobacillus*, *Pediococcus*

Introduction: Probiotics aimed at restoration and maintenance of a healthy gut microflora offer potential as a means of controlling enteric pathogen carriage in pigs. Competitive exclusion cultures are particularly effective in pigs (Nisbet, 2002). However, uncertainty regarding their exact composition has led to concerns that they may result in pathogen transmission and could also hinder regulatory approval. Alternatively, defined probiotic strains, most commonly lactic acid bacteria, *Bacillus* or yeast, can be used as feed additives, either individually or as mixtures. However, commercial animal probiotic products do not always contain the strains or species listed on the label at an adequate dose or may lack evidence of a probiotic effect (Weese, 2002), highlighting the need for proper

selection and characterisation of strains intended for probiotic use. Furthermore, although interest is increasing in the use of probiotics as microbial feed additives for pathogen reduction in pigs, relatively few strains have demonstrated efficacy *in vivo*. The objective of this study was to investigate intestinal performance of five potentially probiotic porcine caecal isolates with anti-*Salmonella* activity (Casey *et al.,* 2003), when orally administered both individually and as a five-strain combination.

Materials and Methods: Twenty eight pigs weaned at 24-28 days were blocked on sex and weight and assigned at random to one of seven treatments (n=4): (1) control (no culture), (2) *Lb. salivarius* DPC6005, (3) *P. pentosaceus* DPC6006, (4) *Lb. pentosus* DPC6004, (5) *Lb. murinus* DPC6002, (6) *Lb. murinus* DPC6003 and (7) mixture of all five cultures. Rifampicin resistant (Rif^r) variants of each strain, required for enumeration were administered as skim milk fermentates containing ~10^8 cfu/ml. The feeding trial consisted of three consecutive periods; 10 days baseline (100 ml sterile skim milk supplemented with 0.5 % (w/v) yeast extract per day), 21 days culture administration (100 ml fermentate providing 3 ~10^{10} cfu/day relevant strain(s) for treatments 2-7 and 100 ml lactic acid-acidified sterile skim milk per day for control pigs) and 9 days post-administration (no milk or culture). Animals also had unrestricted access to water and non-medicated creep feed. Faecal samples were obtained during the baseline (day -5), administration (days 3, 8, 15 and 22) and post-administration (day 26) periods. At day 30 two pigs per treatment were sacrificed and the caecal contents sampled. Faecal and caecal samples were homogenised in maximum recovery diluent as 1:10 dilutions, further diluted and pour-plated. MRS agar containing 100 mg/ml of rifampicin (MRS-RIF) incubated anaerobically at 37 °C for 2 days was used to enumerate the administered strains, while *Enterobacteriaceae* were enumerated on violet red bile glucose agar incubated at 37 °C for 24 h. Representative colonies from MRS-RIF plates from faecal and caecal samples were genetically fingerprinted by RAPD PCR as described previously (Gardiner *et al.,* 1998). The data were statistically analysed using Genstat and Tukey's Test was used for separation of means.

Results: During the 21-day administration period all animals excreted 10^5-10^8 cfu/g of the administered strains (Table 1), as confirmed by genetic fingerprinting of representative faecal isolates using RAPD PCR. However, the *Lb. murinus* strains DPC6003 and DPC6002 demonstrated superior survival, yielding the highest mean faecal excretion of all individually administered strains (Table 1) and accounting for 21-24 % of total faecal lactobacilli. The mean counts for both *Lb. murinus* strains during the 21-day administration period (day 3-22) were higher ($P<0.05$) than those of *P. pentosaceus* DPC6006, which had the lowest mean faecal count (Table 1). The mean faecal day 3-22 count of *Lb. murinus* DPC6003 was also higher ($P<0.05$) than *Lb. salivarius* DPC6005 and *Lb. pentosus* DPC6004 (Table 1), both of which represented on average only 1.1-1.3 % of total faecal lactobacilli. Animals fed the five-strain combination excreted high counts of the administered strains and the mean count during the administration period was higher ($P<0.05$) than that of *P. pentosaceus* DPC6006 (Table 1), with both *Lb. murinus* strains or *Lb. murinus* DPC6003 alone predominating, as determined by RAPD PCR (data not shown). Although faecal counts of the administered strains declined once administration stopped, all animals fed the *Lb. murinus* cultures or the strain combination still harboured high levels of the administered strains at day 5 post-administration (Table 1), with the predominating isolates in the mixture-fed animals identified by RAPD as either *Lb. murinus* DPC6002 or DPC6003 or *Lb. pentosus* DPC6004 (data not shown). These treatment groups also harboured highest levels of the administered strains (10^3-10^6 cfu/g) in the caecum 9 days post-administration, with the *Lb. murinus* strains predominating in one of the mixture-fed animals sacrificed and *Lb. pentosus* DPC6004 and *P. pentosaceus* DPC6006 in the other (data not shown). In comparison, strains DPC6006, DPC6004 and DPC6005, when administered individually persisted less well in both the faeces and the caecum.

Table 1. *Mean faecal counts (log cfu/g) of administered strains in pigs fed ~10^{10} cfu/day of skim milk fermentates of each of the porcine cultures singly or as a combination*

Treatment[a]	Day 3	Day 8	Day 15	Day 22	Day 26 (Day 5 post-administration)	Day 3-22 (Administration period)
Lb. salivarius DPC6005	7.08	6.93^{c}	6.60^{ab}	6.37^{ab}	4.15[1]	6.75^{bc}
P. pentosaceus DPC6006	6.56	5.52^{d}	5.39^{b}	5.61^{b}	4.60[2]	5.77^{c}
Lb. pentosus DPC6004	6.81	7.31^{bc}	6.53^{ab}	6.21^{ab}	5.55[3]	6.72^{bc}
Lb. murinus DPC6002	7.04	8.30^{ab}	7.72^{a}	7.66^{ab}	5.39	7.68^{ab}
Lb. murinus DPC6003	7.74	8.38^{a}	8.29^{a}	8.10^{a}	6.45	8.1^{a}
Culture combination	7.57	8.01^{b}	8.22^{a}	7.85^{ab}	7.29[2]	7.91^{ab}

[a,b,c,d] Means within the same column showing different superscripts are significantly different (P<0.05)
[1, 2, 3] Administered strains detected in only two pigs, three and one pig, respectively

On average, when all treatment groups were analysed together faecal *Enterobacteriaceae* were lower (P<0.05) towards the end of the culture administration period (day 15, 22 and 26) than prior to or during the first week (day -5, 3 and 8) (data not shown). However, no significant effects on faecal *Enterobacteriaceae* were obtained when the entire culture administration period (day 3-22) was analysed, probably because counts were highly variable within individual treatment groups and fluctuated throughout the trial. However, at day 15 mean faecal *Enterobacteriaceae* were lower (P<0.05) in animals fed *Lb. murinus* DPC6003 than in animals fed *Lb. murinus* DPC6002 or the culture mix (Table 2). Furthermore, when mean pre-administration counts were compared with counts after 21 days of culture administration, 87-98 % reductions in *Enterobacteriaceae* were obtained in pigs fed *Lb. salivarius* DPC6005, *P. pentosaceus* DPC6006, *Lb. pentosus* DPC6004 and the culture mix (Table 2), although the results were not statistically significant. Counts also decreased by 83 % in the control group fed acidified skim milk.

Table 2. *Effect of culture administration on mean faecal Enterobacteriaceae counts (log cfu/g) in pigs*

Treatment	Day -5 count (Pre-administration)	Day 15 count	Day 22 count (After 21 days culture administration)	% Reduction after 21 days culture administration[1]
Control	7.39	6.21^{ab}	6.63	83
Lb. salivarius DPC6005	7.42	5.71^{ab}	5.67	98
P. pentosaceus DPC6006	6.87	6.06^{ab}	6.0	87
Lb. pentosus DPC6004	8.10	6.26^{ab}	6.58	97
Lb. murinus DPC6002	6.24	7.31^{a}	6.59	0[2]
Lb. murinus DPC6003	6.56	5.21^{b}	6.69	0[2]
Culture mix	7.22	6.94^{a}	5.74	97

[a,b] Means within the same column showing different superscripts are significantly different (P<0.05)
[1] $(N_0\text{-}N/N_0) \times 100$, where N_0 = mean day -5 count and N = mean day 22 count (both cfu/g faeces)
[2] Counts increased in these treatment groups

Discussion: Survival during intestinal transit and colonization of the gut are important criteria for orally administered probiotics. The strains isolated in our laboratory performed well, especially *Lb. murinus*, which accounted for a large percentage of total faecal lactobacilli, in comparison with previously administered lactobacilli (24 % *vs.* 2.5 %) (Pedersen *et al.,* 1992). The strains administered persisted, albeit to varying degrees, in the faeces and the caecum for at least 5 and 9 days post-administration, respectively, which compares well with previous reports of 3-7 days faecal persistence for other lactobacilli (Pedersen *et al.,* 1992). These findings demonstrate that the porcine isolates compete effectively with the native microflora and become established in the intestine to some extent. The strain variation with respect to survival and persistence in the porcine gut has previously been observed (Pedersen *et al.,* 1992), suggesting that there may be advantages to feeding a culture mix. Indeed, individual strains within

a mix survived differently in individual animals and in general, the culture combination resulted in good survival and persistence, as previously observed by Pedersen *et al.* (1992). Many studies have reported reductions in intestinal coliform and *Enterobacteriaceae* due to probiotic administration; however, some have seen no effects (Simon *et al.,* 2003). In the present study most of the cultures resulted in reductions in faecal *Enterobacteriaceae*; in fact, reductions of up to 98 % were observed. However, except for day 15, these reductions were not statistically significant, probably due to the variation in counts between individual animals, a common observation in probiotic animal trials. This inconsistency may be explained by individual variations in response to probiotics due to the complexity of the intestine (Simon *et al.,* 2003). Future experiments using larger treatment groups and deliberate *Salmonella* infection should provide further information on the pathogen-lowering ability of these cultures.

Conclusions: Pig-derived potentially probiotic cultures with anti-*Salmonella* activity can be effectively delivered to the porcine intestine by oral administration, either individually or as a strain combination. However, it was evident that certain cultures survived at higher levels, persisted for longer in the caecum post-administration and were more effective in reducing pathogenic indicator species, highlighting the advantages of using combination probiotics in pigs. We conclude that, although further characterisation of efficacy is necessary, the findings provide a basis to further explore the potential of these porcine isolates as microbial feed additives (most likely administered as a mixture) for *Salmonella* reduction in pigs.

Acknowledgements: This work was supported by the Irish Government under the National Development Plan 2000-2006.

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Survival of *Salmonella* serovar Typhimurium inside porcine monocytes is associated with complement binding and suppression of the production of reactive oxygen species

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Summary: Macrophages are thought to play a major role in the development of *Salmonella* carriers in swine. It was the aim of the present study to characterize the interactions of a *Salmonella* serovar